Analysis of bacterial DNA from effluent samples for hydrogen production by dark fermentation

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INTRODUCTION

The biological production of hydrogen is attracting more attention due to the utilization of biomass from waste such as effluents. The efficient production of hydrogen highly depends on the bacteria and their ability to degrade organic substrates used as raw material in the bioreactor. Hydraulic retention time (HRT) is one of the critical factors affecting the long-term operation of hydrogen productivity and substrate conversion efficiency of the bacteria under dark fermentation conditions. The study aims to analyze the bacteria involved in the production of hydrogen and optimize the HRT to enhance bacterial growth and its interaction with the substrate to increase hydrogen yields.

METHODOLOGY

Dark Fermentation

Four powdered inoculums (R1 to R4) with known microbes (see table 1, 7) were used in the bioreactors for dark fermentation. A substrate of mixed sugars (lactose, fructose, and sucrose) was used in all four bioreactors with all three HRT conditions of 10 h, 7 h and 7 h using plastic hollow balls as synthetic matrices to immobilize microbes.

Analysis of Bacteria

The bacterial extraction was carried out by filtering the effluent samples, with a 0.45 μm filter. The residues were collected, and genomic bacterial DNA was extracted using the NZYtech soil DNA kit. The quantification of DNA was performed using a Qubit fluorometer.

RESULTS

The extracted DNA was amplified using a 16S barcoding KIT 1-24 (SQK-16S024, Oxford Nanopore Technologies) and sequenced using MinION technology (Oxford). The sequences were obtained by MinKNOW software (Oxford) and demultiplexed into .fastq files to be analysed in EPI2ME agent software (Oxford). The taxonomic tree with sequences were obtained by MinKNOW software (Oxford) and demultiplexed into .fastq files to be analysed in EPI2ME agent software (Oxford). The taxonomic tree with all the bacterial species in the samples was obtained. This enabled the identification of hydrogen-producing bacteria present in the samples.

Table 1: Prominent species observed in the original inoculums and in dark fermentation bioreactors

<table>
<thead>
<tr>
<th>Species</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas fluorescens</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Pseudomonas putida</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Klebsiella aerogenes</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Janthinobacterium lividum</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Clostridium beijerinckii</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

In dark fermentation studies with inoculum R1, Clostridium beijerinckii, Leuconostoc mesenteroides, Klebsiella aerogenes, Janthinobacterium rivuli, Janthinobacterium beijerinckii were observed only at 10 h HRT. Pseudomonas fluorescens, Pseudomonas putida, and Raoultella planticola were observed at 10 h HRT and 7 h HRT with balls indicating that to reduce the retention time, which is the major objective of the analysis, these microbes require synthetic matrices to interact more with the substrate.

CONCLUSIONS

The study has demonstrated the growth pattern of commercial inoculums under diverse environmental conditions at different HRTs. The bacteria observed were either obligate anaerobes or facultative anaerobes except Pseudomonas putida, Comamonas testosteroni, and Pseudomonas filiformis. Some bacteria disappeared when HRT was reduced but some new bacteria were found in the samples. It was also observed that to reduce the HRTs, synthetic matrices like plastic balls can be introduced. The most prominent bacteria observed in the samples include Clostridium species followed by Pseudomonas species.